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Differential Responses of Two Ecologically Similar Case-Bearing Caddisfly Species to a Fish Chemical Cue: Implications for a Coexistence Mechanism

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The mechanisms for the coexistence of multiple species occupying the same ecological niche are often puzzling. Predator effects on competitively superior species is one possible mechanism. In this study, we tested whether the presence of size-selective predators (fishes) acts as a mechanism for the coexistence of two species of case-bearing caddisfly larvae, *Perissoneura paradoxa* and *Psilotreta kisoensis* (Odontoceridae, Trichoptera). The larvae of these two species have similar ecological and life history traits except their body size, and they have been found to coexist only in habitats shared with predatory fishes. Experiments on intra and interspecific competition revealed that the larger *Pe. paradoxa* always outcompeted the smaller *Ps. kisoensis* in the absence of predatory fishes, suggesting that *Pe. paradoxa* performed intra-guild predation on *Ps. kisoensis*. We also conducted experiments to examine how strongly each of these species responded in terms of case repair with/without a predator chemical cue after their cases were partly dismantled. *Perissoneura paradoxa* exhibited a stronger case repair response in the presence of a predator chemical cue than that exhibited by *Ps. kisoensis*, suggesting that *Pe. paradoxa* is more vulnerable to fish predation, probably because their body size is in the preferred prey range of fishes. We suggest that the presence of predators works in the favor of smaller, subordinate species through size-selective predator effects, enabling these two competitive species to coexist in the same habitat.

Key words: chemical cue, intra-guild predation, competitive exclusion, predator-mediated coexistence

INTRODUCTION

A major challenge in ecological studies is understanding how numerous species are able to coexist and interact. Puzzling is the mechanism behind the coexistence of multiple species sharing the same ecological niche, which is contrary to Gause's law of competitive exclusion (Gause, 1934; Hardin, 1960). Selective predation on a competitively superior species is one of the most prevailing factors that permits the existence of inferior species, as it reduces interspecific competition (Paine, 1966; Taniguchi and Tokeshi, 2004; Koivisto et al., 2007). Thus, evaluating predator effects is important to revealing the mechanisms enabling coexistence.

Research into antipredator behavior in response to chemical cues emitted by predators has provided important clues for understanding the evolutional history of prey species in relation to predators in many organisms, such as snails (Bourdeau, 2012), tadpoles (Hettyey et al., 2015), crayfish (Shave et al., 1994), shrimp (Covich et al., 2009), and insects (Cerezer et al., 2016). In addition, it has recently been shown that protective responses could significantly affect trophic cascades through their non-consumptive effects (Relyea, 2000; Trussell et al., 2006; Reynolds and Bruno, 2013). Thus, a prey protective response can be a useful indicator to estimate even complex trophic and competitive interactions.

In general, the protective responses of prey can be classified as anatomical (e.g., prickles on body surface and bulgy body; Kishida and Nishimura, 2004) or behavioral (e.g. pausing, drifting and taking refuge; Alvarez et al., 2014). Anatomical changes often take a long time to develop and are irreversible. On the other hand, behavioral responses are temporary, but the response is quick and reversible. Protective external structures (e.g., nests, burrows, retreats, and cases) built by organisms can be treated as anatomical traits that are extended by the behavioral phenotype (Boyero, 2011). These intermediate phenotypic traits can be quickly developed and are also long-lasting. Therefore, by evaluating the responses of construction behavior, it may be possible to easily estimate predation pressure on a prey species.

The larvae of many caddisfly species (order Trichoptera) construct portable cases in a variety of forms and shapes using materials derived from the sediment (Wiggins, 2004). Some species of case-bearing caddisfly are known to immediately switch their case material from soft material to a more rigid material under the presence of predators (Boyero et al.,

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2006; Boyero, 2011). In addition, these studies have reported that the switching rate of case material and the rapidity of case construction varied depending on the predator species. This indicates the possibility that we can estimate the potential predation risk to a caddisfly species by evaluating case-building responses.

The larvae of two case-bearing caddisfly species, *Perissoneura paradoxa* McLachlan and *Psilotreta kisoensis* Iwata (Odontoceridae, Trichoptera), are widely distributed on Honshu Island, Japan. While their biology is quite similar, there are clear differences in body size where the dry weight of *Pe. paradoxa* is approximately four times that of *Ps. kisoensis*. In this study, we first show that these two species can coexist in a shared habitat only when under predatory pressure. We predict that the size-selective predator effect of fish can be a proximate factor in maintaining the stable coexistence of these two caddisfly species. To test this prediction, we experimentally analyzed the inferior-to-superior relationship between the two species under a fishless condition, and their differential antipredator behavior in response to a fish chemical cue. The present study provides important insights for better understanding the factors that maintain the stable coexistence of ecologically similar species.

MATERIALS AND METHODS

Study organisms

The genus *Perissoneura* is endemic to Japan (Nozaki et al., 1994). The biology and ecology of this genus are closely similar to those of *Psilotreta*, including a shared habitat (confined to spring-fed and headwater mountain stream pools), life cycle (1–2 years voltine; emergence during May; Okano unpublished), feeding habit (cannibalistic scavenger), behavior of settling in the sediment (Wiggins, 2004), case shape (simple cylindrical case constructed from sediment sand), and criterion of case material choice (Okano et al., 2011). However, the body size of *Pe. paradoxa* is clearly larger than that of *Ps. kisoensis*; the maximum body dry weight is 20 mg in *Pe. paradoxa* and 5 mg in *Ps. kisoensis* (Okano et al., 2011). The two species often exhibit size-dependent cannibalism and intra-guild predation (Okano et al., 2016; Okano unpublished), wherein a larger larva will eat a smaller one by invading its case.

Natural habitat

We surveyed the natural habitat distribution of *Pe. paradoxa* and *Ps. kisoensis* larvae on Honshu Island from 2003 to 2014. In each habitat, the presence/absence of predatory fish was confirmed by visual observation and the kick-net method for 1 h continuously. This method for confirming fish absence was sufficient as larval habitats were typically small streams less than 2 m wide and 30 cm deep. We confirmed the absence of fish at certain sites for a period of at least four years.

Determination of inter-specific competition

To evaluate the interaction between *Pe. paradoxa* and *Ps. kisoensis* in the absence of predatory fish, we measured larval survivability and growth under a shared habitat and separated incubation in a laboratory setting. The incubation conditions (larval localities, individual number, and sediment sand types) are described in Table 1. In mid-May 2014, we collected *Pe. paradoxa* pupae from Mt. Tsukuba (Site 2 in Table 2) and *Ps. kisoensis* pupae from Mt. Maya (Site 4) and Mt. Gozen (Site 5). In the laboratory, each pupal population was separately incubated in a 40 × 45 × 20 cm aquarium tank. We obtained egg clutches from the emerged adults at the end of May (23 clutches from *Pe. paradoxa* from Site 2, 7 from *Ps. kisoensis* from Site 4, and 13 from *P. kisoensis* from Site 5). Next generation larvae hatched between June 10 and 14 and were incubated for two weeks in 28 × 20 × 8 cm containers, paved with natural sediment sand from Site 5, and submerged in aquarium tanks. We started the incubation experiment on June 28. Immature larvae of each population were randomly divided into 13 × 13 × 5 cm containers according to the setting conditions. Either of the two species was introduced into a container for the separated condition and both species were introduced together into a container for the sympatric condition. We placed one of two types of artificial sand (70 ml of glass or ceramic sand) in each container, prepared according to previous studies (Okano et al., 2011; Okano et al., 2012). Using the artificial sand as case material, larvae enlarged their cases by extending the anterior end. Rough surface ceramic sand (microscale roughness, i.e., surface texture) is less preferable than smooth surface glass sand for case material, as the rough inner wall decreases larval respiration efficiency by increasing the friction between the abdomen and the case wall, which reduces their growth and increases their metabolic cost and mortality potential (Williams and Pennak, 1980; Okano and Kikuchi, 2009; Okano et al., 2016). Thus, we used the two types of sand to examine the physiological effects on competition based on case material quality. These containers were submerged in a large aquarium tank. During the incubation period, each larva was fed 0.02 mg of fish meal (Tetra Fin; Tetra Co., Melle, Germany) once every three days. We did not adjust the food amount according to the decreasing number of individuals. On 30 July, we measured larval survivability and aperture diameter (AD) of the anterior end of the case in each container. AD data were transformed to larval body weight (DW mg) using the relationship derived from our previous data (Eq. 1 and 2; Okano et al., 2011):

$$\text{Body weight of } Pe.paradoxa = 0.111 \times AD^{2.96} \ (n=119,\ r^2=0.804) \quad \text{(Eq. 1)}$$

$$\text{Body weight of } Ps.kisoensis = 0.323 \times AD^{2.15} \ (n=115,\ r^2=0.620) \quad \text{(Eq. 2)}$$

We considered the difference in mortality between the sepa-

Table 1. Incubation conditions of *Perissoneura paradoxa* and *Psilotreta kisoensis* larvae.

Species	Separated /Sympatric	Individual number	Sediment	Replicate number
Pe. paradoxa	Separated	55	Glass	1
Pe. paradoxa	Separated	55	Ceramic	1
Pe. paradoxa	Separated	110	Glass	1
Pe. paradoxa	Separated	110	Ceramic	1
Ps. kisoensis (Site 4)	Separated	110	Glass	1
Ps. kisoensis (Site 4)	Separated	110	Ceramic	1
Ps. kisoensis (Site 5)	Separated	110	Glass	1
Ps. kisoensis (Site 5)	Separated	110	Ceramic	1
Pe. paradoxa + *Ps. kisoensis* (Site 4)	Sympatric	55:55	Glass	1
Pe. paradoxa + *Ps. kisoensis* (Site 4)	Sympatric	55:55	Ceramic	1
Pe. paradoxa + *Ps. kisoensis* (Site 5)	Sympatric	55:55	Glass	1
Pe. paradoxa + *Ps. kisoensis* (Site 5)	Sympatric	55:55	Ceramic	1

Table 2. Presence or absence of *Perrissoneura paradoxa* and *Psilotreta kisoensis* larvae and predatory fish in a natural habitat.

Site name	Site number	Lat/Long	*Pe. paradoxa*	*Ps. kisoensis*	Fish	Major fish species
Mt. Aoba	Site 1*	38°15'N/140°49'E	×	○	×	
Mt. Tsukuba	Site 2	36°10'N/140°7'E	○	×	×	
Mt. Yokone	Site 3	38°4'N/139°43'E	×	○	×	
Mt. Gozen	Site 5	36°32'N/140°19'E	○	×	×	
Mt. Gozen	SSS of Site 5**	(600 m from Site 5)	×	○	×	
Sekigahara	Site 6	35°22'N/136°27'E	×	○	×	
Sekigahara	1.SSS of Site 6	(85 m from Site 6)	○	×	×	
Sekigahara	2.SSS of Site 6	(900 m from Site 6)	○	×	×	
Kakita	Site 7	35°6'N/138°54'E	○	×	○	amur minnow, sculpin, goby ***
Mt. Maya	Site 4	34°43'N/135°11'E	○	○	○	dark chub sculpin, goby
Hiramizo	Site 8	35°82'N/139°19'E	○	○	○	char, landlocked salmon ****
Akashio	Site 9	35°87'N/137°67'E	○	○	○	char

* Site1-7 correspond to site number in Okano et al. (2011).
** 'SSS of Site5' means habitat where is same stream system of Site5.
*** T. Nozaki (personal communicatin)
**** Yokota et al. (2013)

rated and sympatric-rearing conditions as larval death caused by intra-guild predation among the two species.

Determining predator-mediated competition

To evaluate the interaction between predatory fish and *Pe. paradoxa* and *Ps. kisoensis*, we conducted case repair experiments under the presence/absence of predator chemical cues in a laboratory setting. On August 13, 2014, we collected *Pe. paradoxa* (n = 9) and *Ps. kisoensis* (n = 10) larvae and the predatory white spotted char (*Salvelinus leucomaenis* n = 1) from the Akashio River (Site 9 in Table 2). River water was collected in two plastic bags at the same time. *S. leucomaenis* was placed in one of the bags for 4 h, and we used this water as the source of predator chemical cues to induce the protective behavior among the caddisflies.

In the laboratory, the length and AD of the anterior end of the larval cases were measured under magnification. Larvae were then grouped for two experimental treatments so as to prevent an uneven larval size. The first treatment represented the absence of predatory pressure (n = 5 each for *Pe. paradoxa* and *Ps. kisoensis*) and the second represented the presence of a predator (*Pe. paradoxa* n = 4 and *Ps. kisoensis* n = 5). For all caddisfly larvae used in this experiment, the anterior portion of the larval case was removed (one-fourth of the case length) to induce case re-construction. Each larva was separately placed in a container measuring 3.5 cm in diameter and 1 cm in depth and 2 ml of artificial glass sand was provided as case material. Following this, 10 ml of river water was added to containers of the control (representing predator absence) and 4 ml of river water plus 6 ml of water from the bag that had contained *S. leucomaenis* was added to containers of the treatment condition (representing predator presence). All containers were placed under the eaves of the laboratory building. The experiment started at 6:00 PM on 13 August and ended at 4:00 PM the next day (22 h), by which time the larvae had almost ceased their case repairs. Although the experiment was conducted in a lentic condition, we believe that it closely mimicked the near-natural condition, as their microhabitat is typically limited to sluggish flow areas where chemical cues could accumulate. After the experiment, we measured the amount of glass sand that larvae had used for case repair after preserving them in 100% alcohol (Fig. 1). The amount of glass sand used to repair cases reflected the strength of their response to predatory pressure.

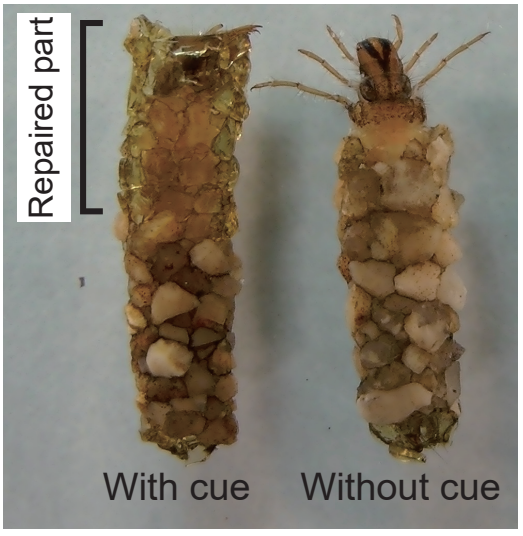

Fig. 1. *Perissoneura paradoxa* after the case repair experiment. Right-side image, larva not exposed to a predator cue and no sand was added after removing a part of case. Left-side image, larva exposed to a predator chemical cue and it repaired its case faster on adding more sand.

Statistical analysis

To assess the sympatric effect on larval survivability, we used a generalized linear mixed model (GLMM) analysis with binomial distributions and a logit link using the incubation container as a random effect (lme4 package, R 3.1.0). We compared binominal data of the number of survivors after one month against the starting number between the sympatric and separated incubation experiments. We also considered the effects of incubation sediment type (glass or ceramic), initial individual number introduced to containers (55 or 110 for *Pe. paradoxa*), and population locality (Site 4 or 5 for *Ps. kisoensis*). The statistical significance of each correlation model was tested by calculating the deviance of the model with and without explaining the terms. The deviance was assumed to have a chi-square distribution. In a similar fashion, to assess the sympatric effect on larval growth, we used GLMM analysis with gamma errors and a logit link using the incubation container as random effects. Explanatory variables were the same as in the survivability analysis.

To assess the effect of predator chemical cues on larval case repair, we failed to fit the data to GLMM because of the zero-inflated data of glass sand number used by *Pe. paradoxa*. Thus, we used a Student's t-test to detect differences in length and AD of the anterior end of the case and the amount of glass sand that larvae used for case repair.

RESULTS

Larval natural habitat

Table 2 shows the presence or absence of *Pe. Paradoxa* and *Ps. kisoensis* larvae and *S. leucomaenis* in their natural habitat. The caddisfly larvae clearly can only coexist in habitats shared by predatory fish (Site 4, 8, and 9). In contrast, *Pe. paradoxa* and *Ps. kisoensis* existed separately in a fish-free habitat (Site 1, 2, 3, 5, and 6).

Sympatric incubation experiment

Fig. 2A shows the survivability of *Pe. paradoxa* and *Ps. kisoensis* larvae under sympatric and separated conditions in the absence of *S. leucomaenis*. For *Pe. paradoxa*, survivability significantly decreased when the initial individual number was high (GLMM, $z = -2.47$, $P = 0.022$). However, there were no significant differences in the survivability for this species between the separated and sympatric treatments ($z = 0.458$, $P = 0.65$) and between the two sediment sand types ($z = 1.451$, $P = 0.19$), indicating that this species was not negatively affected by *Ps. kisoensis*. In contrast, survivability of *Ps. kisoensis* significantly decreased in the sympatric treatment (from 86–96% to 7–30%; GLM, $z = -9.059$, $P < 0.00001$), but there were no significant effects caused by sediment sand type ($z = 0.815$, $P = 0.415$) and population locality ($z = 0.202$, $P = 0.840$), indicating that this species was negatively affected by *Pe. paradoxa*.

Fig. 2B shows the body weight of *Pe. Paradoxa* and *Ps. kisoensis* larvae after one month incubation. For both species, larval body weight was higher in the sympatric condition than in the separated condition (GLMM, *Pe. paradoxa*: $t = 7.505$, $P = 0.00028$; *Ps. kisoensis*: $t = 10.33$, $P < 0.00001$) and higher in glass sediment than in ceramic sediment (*Pe. paradoxa*: $t = 2.786$, $P = 0.021$; *Ps. kisoensis*: $t = 5.20$, $P = 0.0044$). For *Pe. paradoxa*, body weight was significantly lower when the initial individual number was high ($t = -4.881$, $P = 0.0030$). For *Ps. kisoensis*, there were no significant effects of population locality ($t = 1.17$, $P = 0.24$).

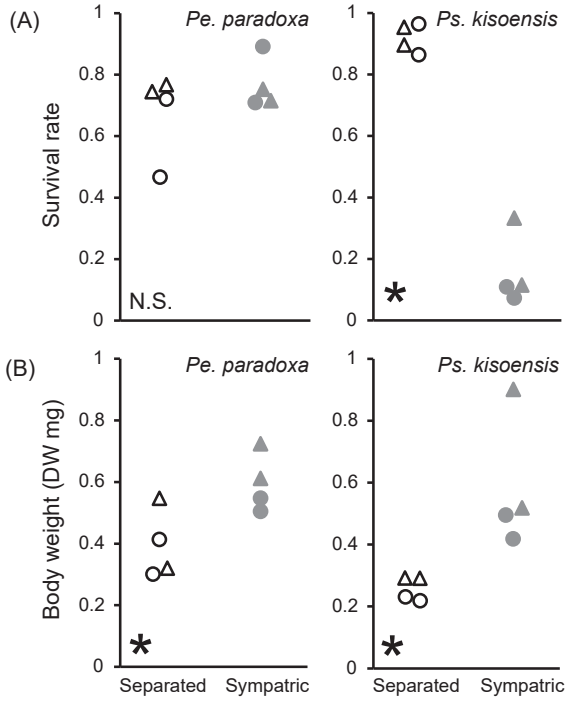

Fig. 2. Survivability **(A)** and body weight **(B)** of *Perissoneura paradoxa* and *Psilotreta kisoensis* larvae after 1-month incubation without predatory fish. Open symbol: separated condition, Grey symbol: sympatric condition, Triangle: smooth glass sediment condition, and Circle: rough ceramic sediment condition. Each circle corresponds to each incubation deme (mean body weight of a deme is indicated in Fig. 2B).

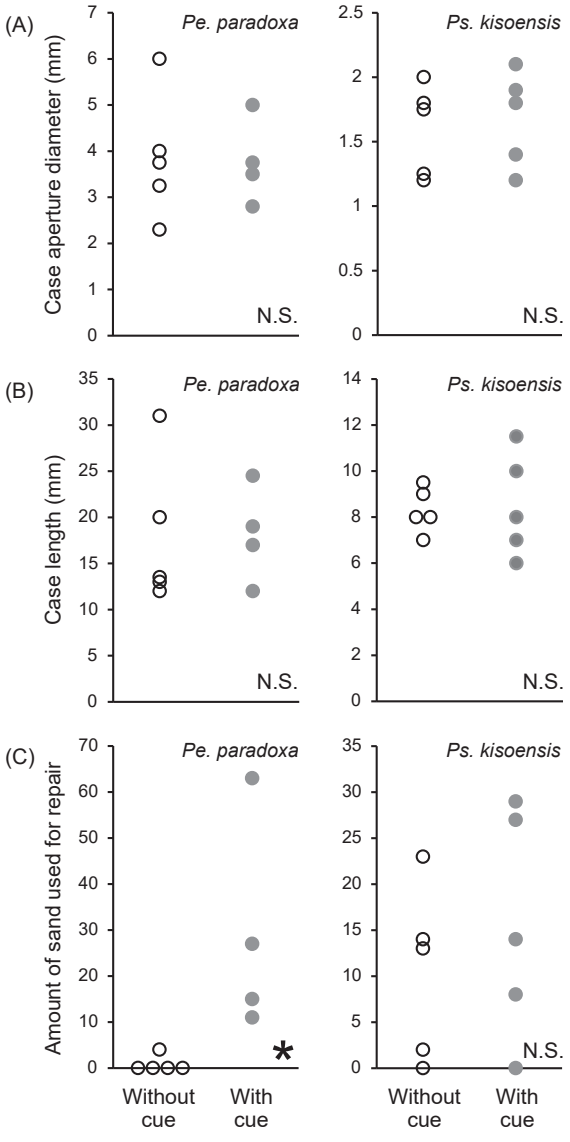

Fig. 3. The length **(A)** and aperture diameter **(B)** of natural cases sacrificed for the repair experiment and amount of glass sand used for case repair **(C)** for *Perissoneura paradoxa* and *Psilotreta kisoensis*. Open circles show incubation without exposure to a predatory fish chemical cue and grey circles show incubation with a cue. Each circle corresponds to each individual.

Case repair in response to *S. leucomaenis*

Figure 3A and B show length and AD of the natural cases sacrificed for the repair experiment. There were no significant differences in case length (t-test, *Pe. paradoxa*: df = 7, t = 1.2, P = 0.45, *Ps. kisoensis*: df = 8, t = 0.35, P = 0.47) and case diameter (*Pe. paradoxa*: df = 7, t = 0.05, P = 0.48; *Ps. kisoensis*: df = 8, t = 0.18, P = 0.43) between *S. leucomaenis* cues and the control treatment.

Figure 3C shows the amount of glass sand used for case repair with *S. leucomaenis* cues and control treatments. For *Pe. paradoxa*, larvae exposed to *S. leucomaenis* cues added a larger amount of sand to their case (df = 7, t = 2.7, P = 0.015). As a result, larva within the *S. leucomaenis* chemical cue treatment clearly enlarged their case compared with those in the control (Fig. 1). On the other hand, *Ps. kisoensis* larvae showed no significant difference in their case repair rate between *S. leucomaenis* cues and control treatments (df = 8, t = 0.75, P = 0.24).

DISCUSSION

The competitive exclusion principle dictates that two species competing for common resources cannot coexist at constant population values if other ecological factors remain constant. However, it has been theoretically and empirically established that size-selective predation can be a proximate factor to maintain coexisting stability (Sprules, 1972; Hall et al., 1976; Kesavaraju et al., 2007; Koivisto et al., 2007; Hülsmann et al., 2011). In the present study, we examined how two ecologically similar species of caddisfly larvae with different body sizes can coexist under the influence of predatory pressure.

In natural habitats, *Pe. paradoxa* and *Ps. kisoensis* existed separately when predatory pressure was absent (Table 2). In particular, at Sites 5 and 6, the two species did not coexist, even within a 1 km area. These habitat segregations strongly indicate that these two species cannot coexist in the absence of predatory pressure. The laboratory experiments support this natural distribution pattern. The smaller *Ps. kisoensis* larvae showed a dramatic decrease in survivability when they coexisted with *Pe. paradoxa*, whereas the survivability of the larger *Pe. paradoxa* did not differ between separated and sympatric incubation. In addition, *Pe. paradoxa* grew faster under the sympatric condition than in the separated condition while maintaining their population. These results indicate that these two species cannot coexist as *Pe. paradoxa* predates *Ps. kisoensis* (Fig. 4A). Furthermore, when larvae were exposed to *S. leucomaenis* chemical cues, the case repair speed was much higher for *Pe. paradoxa* than it was for *Ps. kisoensis* (Fig. 3C). Assuming that the response strength of prey reflects potential predation risks from predators, *Pe. paradoxa* is more vulnerable to predatory fish than *Ps. kisoensis*. Indeed, it was suggested that the brook trout (*Salvelinus fontinalis*) selectively preys upon larger benthic macroinvertebrate species from June to September (Bechara et al., 1992). Taken together, *Pe. paradoxa* strongly preys upon and excludes *Ps. kisoensis* under predator-free conditions. However, under the presence of a fish predator, predatory exploitation weakens as *Pe. paradoxa* is at a higher predation risk from the fish (Fig. 4B). Under such a condition, the two species are able to coexist in the same habitat.

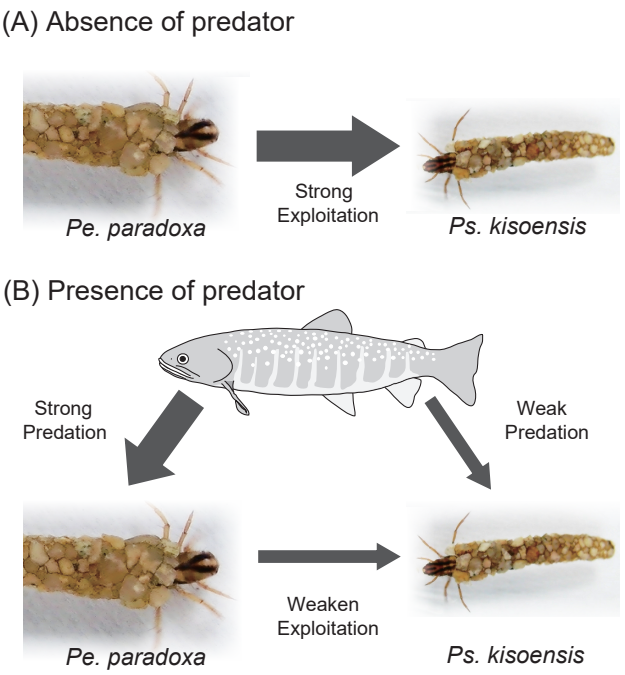

Fig. 4. **(A)** Interaction between *Perissoneura paradoxa* and *Psilotreta kisoensis* without exposure to a predator, and **(B)** possible triangle interactions among them and predatory fish.

Predators can facilitate the coexistence of multiple competitive prey species both through consumptive and nonconsumptive effects of predation (Yurewicz, 2004; Yamauchi and Yamamura, 2005; Aránguiz-Acuña et al., 2010). The former effect appears when highly active, and thus the faster growing preferred prey suffers higher mortality from the predator, while the latter effect appears when the preferred prey ceases to exploit the less-preferred ones so that they do not encounter a top predator (i.e., growth/predation-risk trade-off; McPeek, 2004). In our case study, the extensive exploitation between *Pe. paradoxa* and *Ps. kisoensis* only occurred for two months after they had hatched. In addition, we never observed active *Pe. paradoxa* predation on *Ps. Kisoensis*, as their refractive size difference increased in later life stages, because larger larvae cannot intrude into smaller cases (Okano, unpublished). These observations indicate that whether predatory exploitation occurs or not is determined in the early life stage, before *Pe. paradoxa* grows to the preferred prey size for fish. Assuming that the intraguild predation of *Pe. paradoxa* is associated with high activity, fast growth, and longer periods of risk to predation by fish (i.e., reaching the prey size window of fish earlier), *Pe. paradoxa* may cease its predatory exploitation of *Ps. kisoensis* to avoid fish predation, which permits the coexistence of the two species.

Interestingly, *Ps. kisoensis* also grew larger in the sympatric condition than in the separated condition. It may have been due to minimal resource competition as their population decreased due to predation (Huss et al., 2010). Otherwise, the surviving *Ps. kisoensis* may switch to a fast-growing strategy to escape from the prey size window of *Pe. paradoxa*. Indeed, our unpublished data shows that *Ps. kisoensis* grows larger as the risk of cannibalism increases.

Although there is no concrete evidence to explain why

the competitively inferior *Ps. kisoensis* could exclusively inhabit Sites 5 and 6 without fishes, we think that the reason lies in the difference in case material sizes available at these sites. *Perissoneura paradoxa* use larger sized sand particles as a case material in accordance with their body size difference. Both species need to use smooth surface sand (e.g., quartz mineral) for their case material to promote respiratory efficiency and thus reduce their metabolic costs (Okano et al., 2010; Okano et al., 2016). Indeed, our results show that the body weight of larvae incubated in rough ceramic sand was lighter than that of the larvae incubated in smooth glass sand. However, the abundance of smooth particles in the natural sediment depended on its mineralogical/petrological origin. At Sites 5 and 6, the mineral composition was different between the *Ps. kisoensis* and *Pe. paradoxa* habitats. The sediment of the *Ps. kisoensis* habitat contained relatively smooth minerals (quartz and chert) within the size range that could be used as case material (Okano et al., 2011). In contrast, only the unfavorable rough mineral (sand-mudstone) was evident in the size range used by *Pe. paradoxa*. The sediment of the *Pe. paradoxa* habitat contains smooth surface chert minerals throughout the size range used as a case material (Okano personal observation; the difference of mineralogical component by sediment granularity is described in Okano et al., 2012). Therefore, *Pe. paradoxa* may not be able to invade the neighboring habitat of *Ps. kisoensis* given the unavailability of favorable case material. Although the superiority of *Pe. paradoxa* (i.e., *Ps. kisoensis* survival) did not differ between rough and smooth sand habitats in our sympatric-reared experiment, we may be able to test this possibility by rearing larvae in manipulated habitats, mixing the two sand types and manipulating the smooth sand availability for each size fraction.

Three species of Odontoceridae, *Psilotreta japonica*, *Psilotreta kisoensis* and *Pe. paradoxa*, are recorded on Honshu Island, Japan. Two species with different body sizes (*Pe. paradoxa* and *Ps. kisoensis*) inhabit a similar environment of headwater streams, whereas *Ps. japonica*, which has a similar body size to *Ps. kisoensis*, inhabits lowland streams (Tsuda, 1956; Kawase, 2013). In contrast, in North Korea, two closely related species *Psilotreta falcula* and *Psilotreta locumtenens* that have a similar body size, copulatory organ, and phenology, and often share the same habitat (Nozaki, personal communication). In this case, other than body-size difference, there should be other factors that permit their coexistence. For instance, differences in the activity rate and effectiveness of antipredator behaviors between two competitive limnephilid caddisfly species led to selective predation by the salamander and further induced reversal in the competitive superiority between them in subalpine wetlands (Wissinger et al., 1996; Wissinger et al., 1999). Therefore, a different predator composition may have resulted in the different odontocerid assemblages between Japan and Korea, due to the conflicting requirements of avoiding different predators.

The protective response of a prey to predator chemical cues can be useful to estimate the potential predation risk because the response strength of the prey reflects the underlying connections with other traits related to vulnerability (Dewitt et al., 1999; Boyero, 2011). Indeed, the magnitude of the prey response is often related to predation risk (Eklöv, 2000; McCarthy and Fisher, 2000; Mowles et al., 2011). Although numerous studies have empirically examined protective plasticity with the view of simple bilateral prey–predator interactions or multiple combinations, few studies have considered more than triangular trophic interactions (Relyea, 2000; Trussell et al., 2003). In nature, the responses of prey species are not necessarily reflected at the population level because interspecific interaction is often modified by complex interactions among multiple species. Thus, different predators often create quite different species assemblages (Werner and McPeek, 1994), or extinction of a predator can lead to unexpected cascades of secondary extinctions (Paine, 1966; Sanders et al., 2013). Mowles et al. (2011) showed that a predator chemical cue reversed the competitive superiority between two snail species as they differed in the degree of antipredator response depending on their susceptibility. Therefore, the evaluation of response strength among multiple species is important to understanding complex interspecific interactions.

In conclusion, by evaluating the strength of a protective response, we could explain the possible mechanism behind the coexistence of ecologically similar species in natural habitats. Further studies to confirm their actual vulnerability and the effect of a predator cue on their population dynamics will determine the validity of our hypothesis as a mechanism of coexistence. In addition, we demonstrated the possibility that the coexisting mechanism of odontocerid species in North Korea differed from that in Japan. *Psilotreta* spp. are widespread throughout East Asia and they have many common ecological characteristics (Dudgeon, 1999). Thus, determining the cause of spatial differences among *Psilotreta* spp. and *Perissoneura* provides an excellent model for achieving a better understanding of community structure in relation to speciation and diversification in freshwater ecosystems.

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COMPETING INTERESTS

The authors have no competing interests to declare.

AUTHOR CONTRIBUTIONS

JO: conceived and designed the study, performed all experiments, and wrote the MS. SN: Revised the MS. IT: Revised the MS. NO: Revised the MS.

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